Journal of Feline Medicine and Surgery (2004) **6**, 63–67

jfms

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Evaluation of an in-practice test for feline coronavirus antibodies

Diane D. Addie[a]*, Shona A. McLachlan[a], Matthew Golder[a], Ian Ramsey[b], Oswald Jarrett[a]

[a] *Department of Veterinary Pathology, Institute of Comparative Medicine, Companion Animal Diagnostics, University of Glasgow, Bearsden Road, Glasgow, Scotland G61 1QH, UK*
[b] *Department of Veterinary Clinical Studies, University of Glasgow, Bearsden Road, Glasgow, Scotland G61 1QH, UK*

Accepted 3 December 2003

Summary A commercially available in-practice test for feline coronavirus (FCoV) antibodies (FCoV Immunocomb, Biogal Galed Laboratories) was evaluated by comparison with the gold standard FCoV immunofluorescent antibody (IFA) test. One hundred and three serum or plasma samples were selected and tested: 70 were positive by both tests, 24 were negative by both tests. The in-practice test produced five false positive and four false negative results. The sensitivity of the in-practice test was 95% and the specificity was 83%. When the titres were compared it was found that the in-practice test results were significantly correlated with IFA titres but the degree of correlation was not likely to be clinically useful. The IFA titres of the four false negative samples were found to be low (less than 40) which suggests that even a cat with a false negative result is still unlikely to be excreting FCoV.

A negative result with the in-practice assay is likely to be reliable for screening cats prior to entry into an FCoV-free cattery or stud. It would also be useful in the investigation of suspected FIP as most cats with this condition have high IFA titres of antibodies. A strong positive result would be useful in the diagnosis of FIP (in conjunction with other biochemical and cytological testing), but positive results would be of limited value in monitoring FCoV infection in healthy cats as the antibody titre could not be reliably compared with those obtained with IFA. All positive results obtained using the in-practice kit should be confirmed and titrated by IFA. The kit also appeared to work efficiently with ascites samples (n=6) but too few samples were analysed to draw firm conclusions.

Introduction

Feline coronavirus (FCoV) is a ubiquitous infection of cats that occasionally causes the lethal vasculitis, feline infectious peritonitis (FIP). The measurement of anti-FCoV antibodies is useful in the monitoring of FCoV infections and, when used with other clinicopathological parameters, may assist in the diagnosis of FIP. These parameters include α-1 acid glycoprotein concentration, albumin:globulin ratio, haematology or cytology of effusion (Duthie et al., 1997; Paltrinieri et al., 2001; Sparkes et al., 1994).

FCoV antibodies are often used to screen for the presence of FCoV infections before entry into a breeding cattery or other FCoV-free household. They may also be used to determine the efficacy of early weaning and isolation (Addie and Jarrett, 1990, 1992). In these situations, the measurement

* Corresponding author. Tel.: +44-(0)141-330-5786; fax: +44-(0)141-330-5748
E-mail address: D.D.Addie@vet.gla.ac.uk (D.D. Addie).

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doi:10.1016/j.jfms.2004.01.001

of FCoV antibodies can be more useful than the detection of the virus itself, in that a single serum sample with an antibody titre less than 1:10 indicates that a cat is unlikely to be shedding the virus (Addie and Jarrett, 2001). In contrast it requires five consecutive monthly reverse transciptase polymerase chain reaction (RT–PCR) tests on faeces to demonstrate that a cat is no longer shedding FCoV (Addie and Jarrett, 2001).

Cats with FIP usually have a very high antibody titre to FCoV, so a negative result is useful in ruling out a diagnosis of FIP (Sparkes et al., 1994). However, occasional cats with effusive FIP have low antibody titres on serological tests because their antibody has been bound by the large amounts of virus that are present in the effusion.

A commercial in-practice FCoV antibody test (FCoV Immunocomb®) has recently become available. In the present study this test was compared with the IFA test which was considered to be the gold standard test for measuring anti-FCoV antibodies.

Materials and methods

Immunofluoresence

Immunofluorescent antibody titres were determined as previously described (Addie and Jarrett, 1992). Samples were initially diluted 1:10 in phosphate buffered saline, then dilutions were doubled to 1280. Only half the cells in each well of the plate were infected, giving an internal negative control for non-specific binding of antibody to the cell sheet. Antibody titres of 10 or less were counted as seronegative, 20 or more as seropositive. Titres of greater than 1280 were considered as 1280.

Test kits

A commercially available enzyme immunoassay kit (Immunocomb FCoV (FIP) Anitbody Test Kit, Biogal Galed Laboratories, Israel) was used. These kits contain combs of test strips: each test strip has three reaction areas: a positive control, a negative control and the test area (see Fig. 1). The kits were stored in a refrigerator at 4 °C, as per the manufacturer's instructions. Five kits used were from one batch and 5 kits were from a second batch. The kits were used according to the manufacturer's instructions. The kits were brought to room temperature before use. The samples to be tested were thawed to room temperature. Five microlitres of sample were added to each sample well, and the test was run as per the manufacturer's instructions. The comb was dipped in a series of wells for specific lengths of time, and agitated vertically every 2 minutes. When the reaction was completed the comb was allowed to dry before being read by comparison with a colour scale supplied with each kit (Fig. 2). A reading of 1 to 6 was obtained by comparing the shade of grey of the dot with that of the card. As this is a subjective assessment, two independent readers were used. The positive control dot was aligned with the reading of 3, so that readings of 3 or over were considered positive, readings of 2.5 or less were considered negative. The manufacturers classification of 'low positive' for values greater than 0 but less than 2.5 was not used as early experiences with the kit had suggested that these were usually negative on IFA. Dots that appeared darker than level 6 were counted as 6.

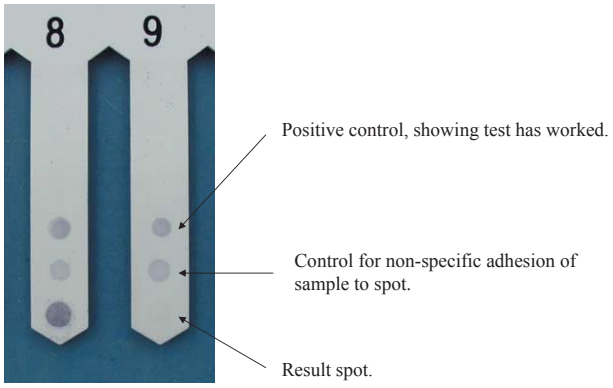

Figure 1 Two Immunocomb teeth showing the three spots: positive and negative control spots and the sample one. Number 8 is positive, number 9 is negative.

Samples

One hundred and three samples of plasma or serum and six ascites were selected from samples submitted to a diagnostic laboratory by practising veterinary surgeons for FCoV antibody testing. The samples were chosen such that FCoV IFA titres ranged from less than 10 to over 1280. Twelve samples were paired blood and ascites taken from six cats. All were stored at −20 °C between IFA testing and use in this study.

Data analysis

Sensitivity and specificity were determined by standard calculations and quoted to the nearest integer (Addie and Ramsey, 2001). Positive and negative predictive values were not calculated as these samples were selected and therefore not

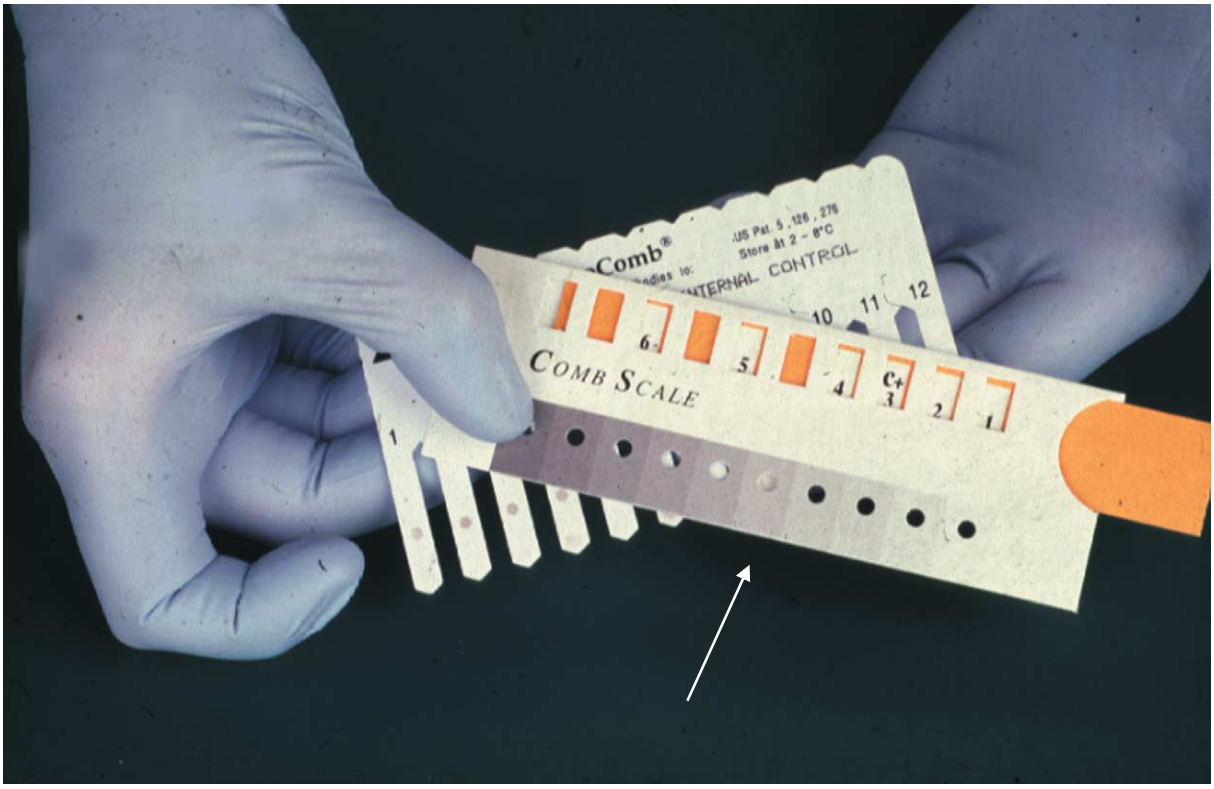

Figure 2 The result dot is read by first setting the comb scale control (C3+) with the negative control spot, then matching the colour of the test spot to the comb scale. On this sample (white arrow), the result is 4.5.

representative of the population. The Pearson correlation coefficient between the two readers was calculated using a standard software package (Minitab v13 for Windows, Minitab Inc). The average of the two readers was used for subsequent analyses. The correlation coefficent between the average of the two readers and the logarithmic value of the IFA titre was then calculated.

Results

The correlation between the two readers was 0.95 (P<0.001) and so an average of the two readings was used for the remainder of the calculations. Only three samples were scored as positive by one reader and negative by the other (with no consistent pattern). The average reading in all three cases was positive and so this was the result that was recorded. Seventy samples were positive by both the IFA and the Immunocomb, 24 were negative by both tests (see Fig. 3). Five samples with IFA titres of 10 or less (i.e. negative) gave positive Immunocomb results. Four Immunocomb negative samples had positive IFA titres. Of these four, three had an IFA titre of 20 and one had an IFA titre of 40. No sample with an IFA titre of over 80 was missed by the Immunocomb. The sensitivity was calculated to be 95% and the specificity to be 83%. Two of the three samples in which the readers disagreed were found to be false positives when tested by IFA.

	Immunocomb Positive	Immunocomb Negative
IFA Positive	70	4
IFA Negative	5	24

Figure 3 A table comparing the positive and negative results obtained with 103 blood samples tested with both IFA and Immunocomb.

A comparison of the IFA titres and the Immunocomb results is shown in Fig. 4. The Pearson correlation coefficient between the average results of the Immunocomb test obtained by the readers and the logarithm of the IFA test was 0.89 (P<0.001).

No formal statistical analysis was undertaken with the six samples of ascitic fluid as there were too few samples, however, the results appeared to be very similar to those found with blood (see Fig. 5).

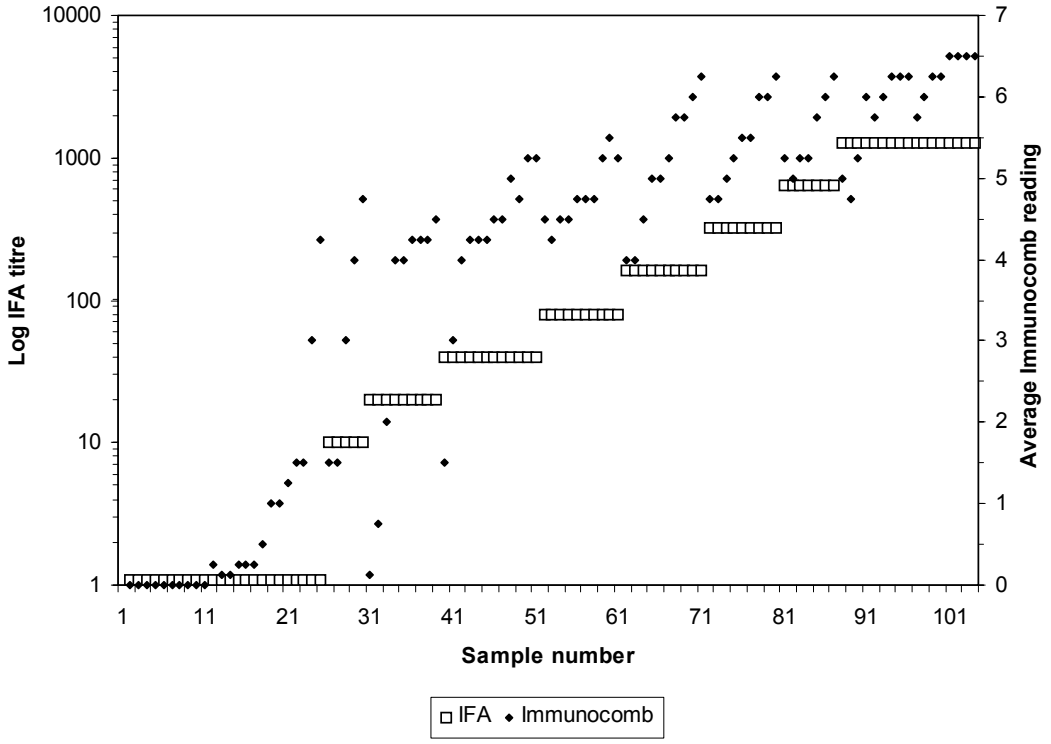

Figure 4 This graph shows the average Immunocomb result of the two readers and the logarithm of the titre obtained using the immunofluorescent antibody (IFA) test for each of the 103 blood samples.

Discussion

From our data it is clear that the FCoV Immunocomb compares favourably with the IFA test and may be useful in some situations in veterinary practice. However, as positive and negative predictive values could not be calculated so the true value of this kit to the practitioner requires further analysis. The value will vary according to the prevalence of FCoV in the practice and the intended use of the kit. The most useful application of this test is likely to be in the screening of cats before entry into FCoV-free households. As the false negative cats had low IFA titres and these are associated with a relatively low prevalence of virus shedding it therefore follows that the false negative results are less of a problem. We would not recommend putting an Immunocomb positive cat into a FCoV-free household as there is approximately a 33% risk that he or she will be excreting virus (Addie and Jarrett, 2001). The Immunocomb only missed 4 of 81 seropositive samples. Thus the Immunocomb is an adequate screening test for testing cats for entry into FCoV free households.

Immunocomb results may also be of some value in the diagnosis of FIP. Although serology alone should never be used to confirm or refute the diagnosis of FIP, negative titres can suggest a

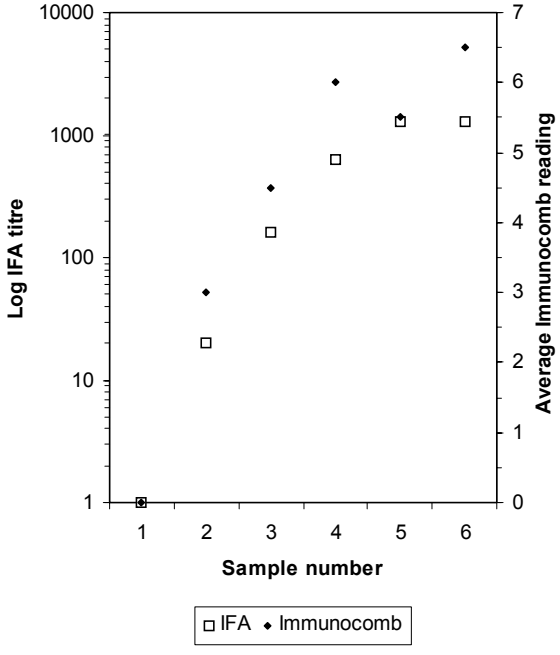

Figure 5 This graph shows the average Immunocomb result of the two readers and the logarithm of the titre obtained using the immunofluorescent antibody (IFA) test for each of the six ascites samples.

reappraisal of the case and high titres can be a useful adjunct to diagnosis. In view of the occurrence of false positive Immunocomb results, all positive Immunocomb results in sick cats require further investigation using an IFA test. In contrast, a negative result is more useful as it reduces the likelihood that the cat has FIP as the cat either has no or very low levels of antibodies when assessed by IFA.

Immunocomb results will not be useful in monitoring cats for loss of FCoV antibodies after they have eliminated FCoV infection. This is because of the poor correlation and the inability to clearly distinguish between high and low or moderate titres. For example, the finding that an IFA titre fell from 1280 to 320 to 40 over a period of time would indicate that the cat was in the process of eliminating a FCoV infection and should be isolated from sources of re-infection. The Immunocomb could not detect that change. IFA is recommended for this purpose.

The trial failed to simulate the situation in veterinary practice in two features. Firstly, in practice it is likely that only one cat would be tested at a time, whereas in this trial all 12 tests were used in a single session. It was not determined if the kit would still be as reliable after repeated warming and chilling. Secondly, the use of whole blood was not assessed (although the manufacturer claims that it works).

In conclusion, the performance of the FCoV Immunocomb compared favourably with the IFA test and it should be useful in the investigation of suspected FIP cases and in screening cats before entry into FCoV-free households. However, caution should be exercised in attempting to correlate its titres with those obtained by IFA.

Acknowledgements

The authors would like to thank Joyce Simpson and Michael McDonald for technical help, Maria Williams and Janet McGrane for secretarial help and Drew McConnell, Graeme McCombe and Andrew Gordon for computing help. We are grateful to Biogal Galed Laboratories and Companion Animal Diagnostics for funding the project.

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